67

THE SHAPE OF THE TARGET MOLECULE AND THE DIFFUSION DISTANCE OF RADICALS FORMED BY IONIZING RADIATION

S. B. BHATTACHARJEE AND N. N. DAS GUPTA

BIOPHYSICS DIVISION,

SAHA INSTITUTE OF NUCLEAR PHYSICS,

CALCUTTA-9, INDIA.

ABSTRACT. The increased sensitivity of any micro-organism, when irradiated in wet condition, is explained by the radical formation in the film of water surrounding the vital molecules and the diffusion of these radicals to the vital molecules causing their inactivation. This diffusion distance can be obtained from a measurement of the doses of radiation for inactivation in the dry and wet conditions. This distance is a function of the shape of the vital molecule, the number of effective radicals per primary ionization and the ratio of dry to wet irradiation doses. In this paper is reported a simple method for estimation of this diffusion distance for an ellipsoidal target of any axial ratio for any measured value of dry and wet irradiation doses.

INTRODUCTION

Biological material is more sensitive to radiation in the wet than in the dry state of irradiation. This additional inactivation of biological samples, when irradiated in hydrated condition, is due to the formation of radicals in the water surrounding the vital molecules, some of which subsequently diffuse to these and inactivate them (Zirkle and Tobias, 1953; Hutchinson, 1957; Hutchinson and Ross 1959; Hutchinson and Norcross, 1960). The diffusion distance in the different cases has been estimated on the assumption of a spherical target. In a recent paper Hutchinson and Arena (1960) have calculated the diffusion distance in the case of irradiation of DNA *in vivo*. They assumed the model of a right cylinder.

In the present report, a simple theory has been developed for estimation of the diffusion distance for the general case of ellipsoidal target molecule. This method enables one to determine the diffusion distance for any measured values of dry and wet irradiation doses, for any axial ratio and for different probabilities of inactivation by radical formation.

This theory has been used to calculate the diffusion distance from the experimental data on dry and wet inactivation of different enzymes as presented by previous workers.

ANALYSIS OF INDIRECT EFFECT

If the inactivation is due to a single-hit type of process the survival ratio $S = N/N_0$ after dose of D rads is given by

$$S = \exp(-I\sigma V) \quad \ldots (1)$$

where I is the number of primary ions per cc of unit density material produced by the dose D rads and V is the volume of a target molecule responsible for inactivation. Assuming that 1 rad of energy absorption releases $5.68 \times 10^{11}\sigma$ primary ions per cc of the target material of density σ, the total target volume V is given by

$$V = \frac{1}{D_d \times 5.7 \times 10^{11}\sigma} \text{ cc} = \frac{1.76 \times 10^{12}}{D_d\sigma} \text{ Å}^3 \quad \ldots (2)$$

where D_d is the dose of radiation giving 37 per cent survival in the dry condition.

If the target molecule be assumed as an ellipsoid of revolution with the semi-axes a and b, the axis of revolution being the b axis, the volume V is given by

$$V = \frac{4\pi}{3} ab^2 = 4.18\rho b^3 \text{ Å}^3 \quad \ldots (3)$$

where

$$\rho = a/b. \quad \ldots (4)$$

Combining Eqs. (2) and (3), the dimensions of the target molecule is given by

$$b = \frac{7.5 \times 10^3}{(D_d\rho\sigma)^{1/3}} \text{ Å} \quad \ldots (5)$$

If σ be assumed as 1.33 for protein

$$b = \frac{6.81 \times 10^3}{(D_d\rho)^{1/3}} \text{ Å} \quad \ldots (5a)$$

The effect of hydration is to increase the target dimensions by an additional thickness of water, from which the radicals can diffuse to the target and inactivate it. If it is assumed that in the wet condition of irradiation, each target molecule is surrounded by an uniform layer of water of thickness ρ Å, then the volume of this water film is given by

$$v_\omega = \frac{4\pi}{3}[(a+\rho)(b+\rho)^2 - ab^2] = \frac{4\pi}{3}[\rho^3 + \rho^2 b(2+\rho) + \rho b^2(1+2\rho)]$$

Replacing b from Eq. 5(a)

$$v_\omega = \frac{4\pi}{3}\left[\rho^3 + 6.81 \times 10^3 \cdot \frac{2+\rho}{\rho^{1/3}} \cdot \frac{1}{D_d^{1/3}}\rho^2 + 4.63 \times 10^7 \frac{1+2\rho}{\rho^{2/3}} \cdot \frac{1}{D^{2/3}}\rho\right] \quad \ldots (6)$$

If Y is the number of radicals formed per primary ionization in water and q is the probability of reaction between the radicals and the target molecules, then increase in the reaction probability per primary ionization, due to the presence of this water layer, is given by

$$\Delta V = \frac{4\pi}{3} Yq\left[\rho^3 + 6.81\times10^3 \cdot \frac{2+p}{p^{1/3}} \cdot \frac{1}{D_d^{1/3}}\rho^2 + 4.63\times10^7 \cdot \frac{(1+2p)}{p^{2/3}} \cdot \frac{1}{D_d^{2/3}}\rho\right] \quad \ldots \quad (7)$$

Due to this increase in the sensitive volume, the 37 per cent survival dose in the wet condition is smaller than that in the dry condition and the surviving fraction S is given by

$$S = \exp -I\left[V\sigma + \frac{4\pi}{3} Yq\left(\rho^3 + 6.81\times10^3 \cdot \frac{2+p}{p^{1/3}} \cdot \frac{1}{D_d^{1/3}} \cdot \rho^2 + 4.63\times10^7 \cdot \frac{1+2p}{p^{2/3}} \cdot \frac{1}{D_d^{2/3}} \cdot \rho\right)\right] \quad \ldots \quad (8)$$

where I is the total number of primary ionizations per cc of unit density material and $\sigma = 1.33$.

If D_w and D_d be the 37 per cent survival doses in the wet and in the dry conditions respectively then from Eq. (2)

$$\Delta V = 1.76\times10^{12}\left[\frac{1}{D_w} - \frac{1}{D_d}\right] \quad \ldots \quad (9)$$

$$= \frac{1.76\times10^{12}}{D_d}(K-1) \quad \ldots \quad (10)$$

where $D_d = KD_w$

Equating (7) and (10) and rearranging terms we obtain

$$\rho^3 + 6.81\times10^3 \cdot \frac{2+p}{p^{1/3}} \cdot \frac{1}{D_d^{1/3}} \cdot \rho^2 + 4.63\times10^7 \cdot \frac{1+2p}{p^{2/3}} \cdot \frac{1}{D_d^{2/3}} \cdot \rho = \frac{4.2\times10^{11}}{(D_d Yq)} \cdot (K-1) \quad \ldots \quad (11)$$

$$\text{or } x^3 + 6.81\times10^3 \cdot \frac{2+p}{p^{1/3}} \cdot x^2 + 4.63\times10^7 \cdot \frac{1+2p}{p^{2/3}} \cdot x - \frac{4.2\times10^{11}}{Yq}(K-1) = 0 \quad \ldots \quad (12)$$

where $$x = D_d^{1/3}\rho \quad \ldots \quad (13)$$

This is a general expression giving the values of x as a function of the ratio of the dry to the wet dose (K), the shape factor of the target molecule (ρ) and the probability of inactivation by radical formation per primary ionization (Yq).

This equation may be solved and x obtained for any particular values of K, p and Yq. ρ can then be directly obtained by using Eq. (13) from X and D_{87} in the dry condition. For quick estimation of x under different specified conditions, the charts, given in Figs. 1 and 2, have been prepared.

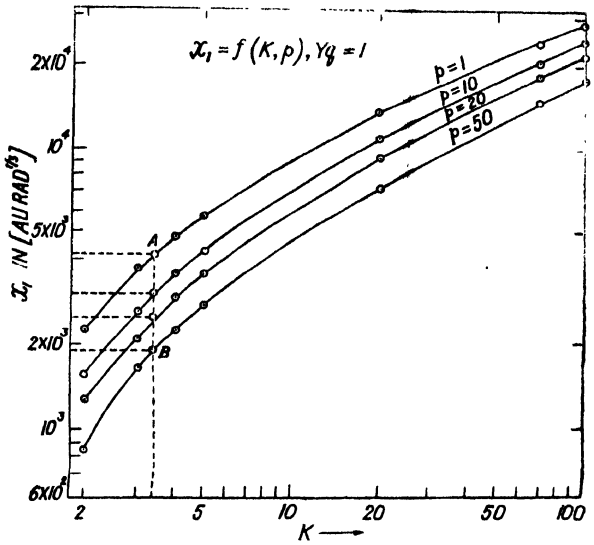

Fig. 1. Variation of x_1 with K, for different values of p.

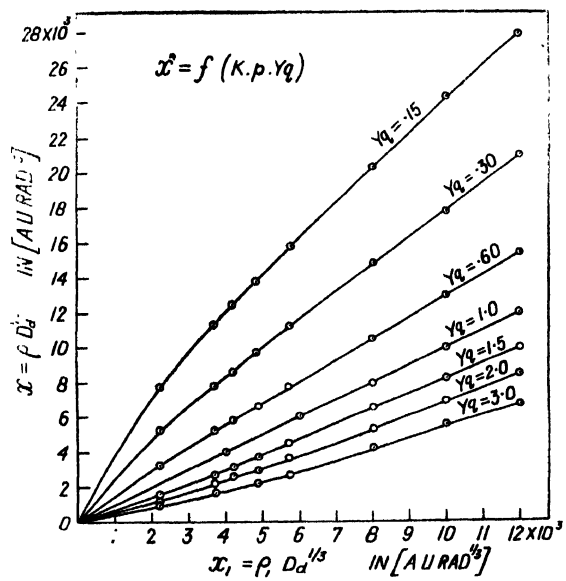

Fig. 2. Variation of x with x_1 for different values of Yq.

ILLUSTRATION

The diffusion distance may be obtained by successive approximations with the help of Figs. 1 and 2. Fig. 1 gives the approximate values of $x = x_1$ for different values of K and p when $Yq = 1$ i.e., the graph is a plot of the equation

$$x_1^3+6.81\times10^3 \cdot \frac{2+p}{p^{1/3}} \cdot x_1^2+4.63\times10^7 \cdot \frac{1+2p}{p^{2/3}} \cdot x_1-4.2\times10^{11}(K-1) = 0 \quad (14)$$

With the help of this graph, x_1 can be obtained for any experimentally measured values of dry and wet doses, when the number of effective radicals formed per primary ionization Yq is assumed to be unity. For example, if the dry dose is 20 kilorads and the wet dose 6 kilorads i.e., $K = 3.3$, the values of x_1 are found to be 4.2×10^3, 3.0×10^3, 2.50×10^3 and 1.88×10^3 for $p = 1$, 10, 20 and 50 respectively as shown by the dotted line AB.

Fig. 2 plots the values of x as a function of x_1, for different values of Yq i.e.- it gives the changes in the values of x_1 to x, as the probability of inactivation per primary ionization changes. It is seen from this curve that in the above example, the values of x_1, obtained previously, change to $x = 3.2\times10^3$, $2.2\cdot10^3$, 1.8×10^3 and 1.4×10^3 respectively as Yq changes from 1 to 1.5. Thus with the help of the graphs given in Figs. 1 and 2, one can obtain the value of $x = D_d^{1/3}\rho$ for any combination of p and Yq.

DISCUSSIONS

The method of estimation of diffusion distance developed here is of a very general nature and may be applied to any ellipsiodal target molecule of arbitrary shape and any assumed value of the reaction probability Yq per primary ionization. If the vital molecule is spherical, diffusion distance can be obtained by assuming $p = 1$.

In the case of inactivation of the enzymes various workers (Rajewsky *et al.*, 1957, Hutchinson, 1960) have assumed spherical model. The diffusion distance calculated from the present method given in column 8 of Table I. Column 7 of the Table I gives the value of diffusion distance obtained previously. Inactivation of DNA *in vivo*, has been investigated by Hutchinson *et al.* (1960) who. showed that D_{37} for the transforming principle in pneumococcus cells was about 3 megarads. Asymmetry of the molecule for the transforming activity was found to be about 40 and diffusion distance calculated from the cylindrical model was 8—10 A.U. The present method gives diffusion distance under this condition as 8 A.U.

The values calculated from the present method seem to be slightly less than those deduced previously. This slight difference is due to the fact that the expression used previously for the calculation of ρ was $\Delta V = 4\pi Yq(\rho b^2+\rho^2 b)$. For $p = 1$, the general expression (7) differs from this by a term involving ρ^3.

This difference arises from the fact that here an average thickness of water film ρ has been assumed around the vital molecule, thus defining a zone in which all radicals formed has equal probability of inactivating the vital molecule. Absence of ρ^3 term in the expression used previously increased the value of diffusion distance in the case of Invertase, ADH, CoA, Cytochrome C. However in the case of TP, the method used considered the ρ^3 term and the agreement between the two values is therefore excellent.

ACKNOWLEDGMENTS

This work was carried out under the financial sponsorship of the Council of Scientific and Industrial Research. One of the authors (S.B.B.) is indebted to C.S.I.R. for a Senior Research Fellowship.

TABLE I

Diffusion distances from the *in Vivo* radiation inactivation of molecules

Sample	D_{37} in megarads.		K	p	Yq per ionization	ρ Previous in AU	ρ Present in AU	Ref.
	Wet	Dry						
TP (DNA)	1.2	3	2.5	40	1.5	8–10	8	H and A (1960)
Invertase	6	12	2	1	0.15	38	33	
Alcohol dehydrogenase	1.3	28	21	1	2.79	31	25	H (1960)
Co-enzyme A	3	200	67	1	2.70	35	25	
Cyto-chrome C	1	54	54	1	0.3	150	105	R. G and P (1957)

REFERENCES

Hutchinson, F. (1957), *Radiation Research*, **7**, 473-483.

Hutchinson, F. and Ross, D.A. (1959), *Radiation Research*, **10**, 477-489.

Hutchinson, F. and Norcross, C., (1960), *Radiation Research*, **12**, 13-19.

Hutchinson, F. and Arena, J., (1960), *Radiation Research*, **13**, 137-147.

Hutchinson, F., (1960), *Amm. Naturalist.*, **94**, 59-70.

Rajowsky, B., Gerber, G. and Pauly, H., (1957), *In Advances in Radiobiology* (G. C. Hevesy, A. G. Forssberg and J. D. Abbatt, Edts.) pp. 25–32, Oliver and Boyd, Edinburgh.

Zirkle, R. E. and Tobias, C. A. (1953), *Arch. Biochem. and Biophys.*, **47**, 282.306.